STUDIES ON THE EFFECTS OF EYESTALK REMOVAL UPON YOUNG CRAYFISH (CAMBARUS CLARKII GIRARD)

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In recent years it has come to be recognized as a result of the work of Megušar (1912), Abramowitz and Abramowitz (1938), Brown and Cunningham (1939), Hanström (1939), and Abramowitz and Abramowitz (1940) that removal of both eyestalks in decapod crustaceans causes the next moult to occur sooner than it would normally. Removal of eyestalks is eventually fatal, as Brown (1938) and Brown and Cunningham (1939) have shown for *Cambarus*, and Abramowitz and Abramowitz (1940) have shown for *Uca*. Brown and Cunningham give evidence, derived from sinusgland implantation, that the factor or factors related to moulting and viability originate in the sinusgland of the eyestalk.

In the above work the general method has been to operate on a number of animals at once, and then to compare the incidence of moulting in this group with that in an untreated group. This procedure gives no information as to the actual amount of shortening of the intermoult following operations, nor has sufficient evidence been presented to prove that the acceleration of moulting is a real shortening of the intermoult and not merely a speeding up of the intermoult in its later stages by the shock of eyestalk removal. The experiments to be described below were intended to clarify these questions by means of operations made after observed moults on animals whose history was known and whose moulting cycles could be followed individually.

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Experimental

In experiments carried out in 1938, young *Cambarus clarkii* were used, ranging in length from 17 to 24 mm. (rostrum to telson). These were kept in individual dishes at 9–14° C. and fed very lightly. Operations were performed two days after animals were observed to have moulted. Eyestalks were occluded by ligating them at the base with fine hairs, while as a control operation antennae were similarly ligated and then removed with scissors. Other animals were left intact as controls.

It was found that removal of both eyestalks was always fatal. In the course of the eyestalk ligations discussed here, and some performed in another connection, 60 animals had both eyestalks tied off. Of these, 53 had died by the close of the experiment. Survival ranged from 1 to 38 days following the operation, averaging 17.1 ± 1.14 [1] days. If deaths within 10 days are disregarded the average survival was 19.7 ± 1.03 days. That this mortality is not due merely to operative injury is shown by the fact that only 2 deaths resulted from antennal removal among 30 animals.

Eyestalkless animals often moulted before dying, with intermoults shorter than in normal animals, but antennal removal had no significant effect upon the length of the following intermoult. Thirty-four crayfish had their eyestalks ligatured after the first observed moult. All died, but 14 moulted before dying, with intermoults averaging 15.9 ± 1.02 days, while in 29 normal animals the average was 28.9 ±1.65 days, and in 30 animals with antennae removed the average was 31.2 ± 2.28 days. This may indicate a significant shortening of intermoult by eyestalk removal, although the results are unsatisfactory because of the high percentage of deaths in the eyestalkless group.

Accordingly, the work was repeated in 1939, using a modified procedure. The essential feature of this second experiment was that operations were made only on animals in which one or more complete intermoults had been observed. Thus an intermoult following an operation could be compared with the preceding "normal" intermoult in the same animal. To hasten growth and moulting the animals were kept at room temperature and were fed liberally, the effort being made to supply as much food as they could eat. This had a marked effect upon survival that will be mentioned below.

Eyestalks were removed, with the animals held on ice, by pinching them off at the base with watchmaker's forceps. As a control operation the severe procedure of cutting off the retinal portion of the eyestalk with fine scissors was used. This caused an open wound with much bleeding. It was found advisable to perform the operations of eyestalk or retinal removal in two steps, taking off the second eyestalk or retina 12 hours after the first. Cautery was not employed.

[1] In this and following cases where standard errors are given, the errors are standard error of the mean, given by the formula $\frac{\text{standard deviation}}{\sqrt{\text{no. of variates}}}$.

The 122 *Cambarus clarkii* used ranged in length from 8 to 12 mm. (rostrum to telson) at the start of the 7-week observation period. Observations were made twice daily. When an animal had moulted twice (one intermoult), it was assigned to one of three experimental groups. The first animal was left as an intact control, the second had both eyestalks pinched off, the third had both retinas cut off. This series of assignments was repeated as moults occurred among the animals. Operations were started 24 hours after an animal was observed to have moulted. Crayfish showing excessively long intermoults were left out in the assignment to groups, but might be admitted later if the next intermoult were near the normal length. After intact controls had moulted a third time they were reassigned to whichever of the three experimental groups they happened to fall into. This resulted in the intact group being depleted of its more rapidly moulting members, which passed into and were recorded with, one of the two operational groups. As a result, the average intermoult for the remaining intact animals is abnormally long, and cannot be compared with the shorter pre-operational intermoults of the other two groups which will be discussed below.

No retinal removals were performed in the last 15 days of the experiment, while no eyestalks were removed in the last 11 days. There were thus more eyestalkless than retinaless animals recorded. A few eyestalkless animals were observed for 3 weeks after regular observations ceased in order to determine their survival times. The complete record of the intermoults observed in the two groups which had eyestalks and retinas removed is given in Tables I and II respectively. The intermoults which will be used to show the effects of eyestalk or retinal removal are the intermoults immediately before and following the operation.

In the group of crayfish whose eyestalks were removed, the intermoults immediately preceding operations averaged 12.13 $\pm$ 0.65 days, while the first intermoults following the operation averaged 8.10 $\pm$ 0.19 days (Table I). That this difference is significant is shown by the fact that the standard error of the difference of the means is 0.68 days, one-sixth the difference of the means. Of the 45 animals operated upon, only one died as a result of the operation, while a second has been disregarded in the calculations of mean intermoult and survival time because it is so obviously at variance with the rest. Twenty-two animals passed through a second intermoult after eyestalk removal. With two exceptions these second intermoults are as low as the first ones after the operations (Table I). Three animals moulted three times in the absence of their eyestalks. These third intermoults were

Table I

Complete record of intermoults observed in the group which had both eyestalks removed, and the survival times of those animals which died naturally following eyestalk removal.

Pre-operational Intermoults (length in days)			Post-operational Intermoults (length in days)			Survival Time (days)
Earlier pre-operational intermoults		Last pre-operational intermoult	First post-operational intermoult	Second post-operational intermoult	Third post-operational intermoult	
		15.5	7.5	7.0		[30–31]
		10.0	8.5	8.5		18.0
		9.5	8.5	8.5		[25–30]
		11.0	8.5			Fixed
	15.5	14.0	8.0			23.0
	11.5	9.0	6.5			Fixed
		11.5	7.0	7.0		Fixed
	10.5	9.0	7.5			Fixed
		11.0	6.5			Fixed
		14.5	7.0			12.5
	16.5	16.5	7.5	8.0	11.5	26.5
		14.5	7.0			Fixed
	10.5	11.0	8.5	9.5		Fixed
		12.5	7.5			Fixed
		12.0	7.5	8.0	10.5	26.0
	[13.0]	[9.0]	[16.0]	[12.0]		[45]
		10.0	7.0	7.0		Fixed
	13.0	11.0	7.0	8.5		[25–30]
		13.0	7.0	7.5	11.0	26.5
		14.0	11.0			19.5
14.0	9.0	10.0	8.5	9.0		[22–27]
		13.5	7.5			Fixed
	14.0	12.0	9.5			9.5
		16.0	7.5	9.0		[16–21]
	14.0	11.5	8.0	9.5		Fixed
		13.0	Died			——
		13.5	12.0			12.0
12.0	8.0	11.0	7.0	[22.0]		28.5
	11.0	[20.0]	8.0	9.0		26.5
		11.0	7.0	8.0		22.5
		10.0	7.5			13.0
		11.0	8.5			Fixed
		16.0	8.5			Fixed
	11.0	11.5	8.0			Fixed
	13.5	11.5	7.5	7.5		[20–25]
	12.0	14.5	7.5	7.5		[22–27]
	14.0	13.0	11.0			11.0
		13.5	8.5			Fixed
	11.0	10.0	7.5	8.0		22.0
		15.0	10.0			10.0
		13.0	8.0	10.0		28.0
	9.5	9.0	8.0			Fixed
	10.5	10.0	10.0			17.0
		10.5	10.0	9.0		18.0
13.0	11.94 ± 1.16	12.13 ± 0.65	8.10 ± 0.19	8.30 ± 0.17	11.0	19.47 ± 1.48

TABLE II

Complete record of intermoults observed in the group which had both retinas removed.

Pre-operational Intermoults (length in days)		Post-operational Intermoults (length in days)		
Earlier pre-operational intermoult	Last pre-operational intermoult	First post-operational intermoult	Second post-operational intermoult	Third post-operational intermoult
	16.5	Died		
12.0	9.0	9.5		
13.0	7.5	7.5		
	13.5	17.5		
15.5	12.5	14.0		
	10.5	17.5		
	15.5	Died		
10.5	8.0	12.5		
	13.0	9.5	9.5	
	14.5	20.0		
15.5	10.5	17.5		
	11.5	16.0		
	10.5	11.5	13.5	
10.0	12.5	10.0	11.5	
	9.5	12.0	13.0	
	13.0	12.5	12.0	15.5
11.0	17.0	12.5		
13.0	13.0	14.0		
	13.0	Died		
11.5	8.0	13.0	9.5	
9.5	11.0	14.0		
11.5	12.0	14.5		
	14.0	16.5		
	16.0	9.5		
	12.5	12.5	11.0	
13.0	10.0	14.0	14.0	
12.0	14.5	11.5		
14.5	8.5	15.5		
11.5	11.0	21.0		
7.5	10.5	18.5		
	13.5	14.5		
	9.5	Died		
	9.5	10.5	9.0	
11.0	10.5	21.0		
11.91 ± 0.50	11.82 ± 1.04	14.02 ± 0.62	11.44 ± 0.58	15.5

longer than the preceding ones, but as eyestalkless animals eat a great deal, and become distinctly larger than normal animals of the same age, this increase is not surprising. The number of second and third intermoults of eyestalkless animals would have been greater had not about a third of the animals been fixed for histological examination at the end of the first intermoult after eyestalk removal.

The effects of retinal removal are in contrast to those of eyestalk removal. With a few exceptions the post-operational intermoults are longer than the preceding ones (Table II). Intermoults in 30 retinaless crayfish average 14.02 ± 0.62 days, while the intermoults just before the operations average 11.82 ± 1.04 days. The increase in the length of intermoult after retinal removal is, however, of doubtful significance, since the difference between the means is only 1.8 times the standard error of the difference. In a few cases a shortened intermoult followed retinal removal. It is difficult to remove completely the retinal zone of the eyestalk without including some ganglionic material, leading one to suspect that in these cases the sinusgland may have been removed as well as the retina. The severity of retinal removal is shown by the fact that 4 deaths resulted from 34 operations. However, the remaining animals continued perfectly healthy, no other deaths occurring during the whole observation period. Retinaless animals did not attain the size of eyestalkless animals, a fact probably related to their slower rate of moulting. They tended to become quite dark in color, in contrast to the eyestalkless animals, which took on a pronounced reddish-brown color, becoming much paler after ecdysis.

Discussion

The results obtained show that eyestalk removal causes a shortening of the following intermoults in young crayfish. The mechanism suggested by Brown and Cunningham (1939) and Hanström (1939), that the eyestalks normally produce a hormone tending to delay or inhibit moulting, seems to be a reasonable explanation.

It has been shown that even severe injury does not shorten the intermoult, at least when the injury occurs early in the intermoult. Darby (1938) has stated that operative injury appears to hasten the next moult in *Crangon armillatus*. Possibly, however, his results may mean that the shock of injury late in the intermoult period causes a speeding up of moulting processes already well advanced in the animal, while injury early in the intermoult may have no such effect. It may be necessary to distinguish between the acceleration of moulting caused by eyestalk removal and that reported by Darby as caused by other types of injury.

The observation of Abramowitz and Abramowitz (1940) that *Uca* may occasionally moult more than once after eyestalk removal has been found to be true also for *Cambarus*, where three intermoults have been recorded after eyestalk removal. It seems beyond question that the processes leading up to moulting can be initiated in the absence of the eyestalks, while "shock" effects are ruled out as a cause of the shortened intermoult.

A comparison of the survival time of eyestalkless animals in the second experiment with that of those in the first reveals a curious discrepancy. In 1938, with cold water (9°–14° C.) and rather scanty food, the average intermoult among eyestalkless animals was 15.9 days, the average survival 17.1, or at most 19.7 days. In 1939, with warm water (20°–22° C.) and unlimited food, the average intermoult in eyestalkless animals fell to 8.1 days, as might be expected, but survival averaged 19.5 days. Although not proved, it seems probable that the abundant food supply increased the expected survival time in 1939 over that in 1938. Otherwise survival in 1939 would have been shortened by warmth and favorable conditions to the same extent that intermoults were shortened. This observation, that in eyestalkless animals shortening of intermoult and time of survival are not necessarily parallel effects, suggests that the processes related to viability in the eyestalkless crayfish may be separated from those processes which underlie the more rapid moulting of such animals. However, no evidence so far presented is sufficient to show whether these processes are controlled by one substance from the eyestalks, or by two or more.

The fact that eyestalkless crayfish may moult successfully shows that the aid of the eyestalks is not indispensable to the physiological changes occurring at ecdysis, but that the moulting processes may be seriously interfered with by lack of the eyestalks is suggested by Abramowitz and Abramowitz (1940), who report that most of the deaths after eyestalk removal follow close upon a moult. This can be verified from the writer's second experiment. Twenty animals were maintained after eyestalk removal until they died. Table III shows that deaths fall into two main groups. The first is directly after the time of moulting, the second about 8 days later. Since the average

Table III

Survival time of eyestalkless crayfish after the last moult

Survival time in days....	0*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Number of deaths.....	2	6	2	—	—	—	2	—	2	2	1	—	1	—	—	—	1	1

* Died in moult.

intermoult of eyestalkless animals is a little over 8 days, it seems probable that the second group of deaths is that of animals which died in an incipient moult, and suggests that the physiological changes associated with moulting impose a severe strain which the animal fails to meet without the aid of some factor from its eyestalks. Presumably the weaker animals die before accomplishing ecdysis, while stronger ones may live long enough to moult, and still others may "recover" and pass through the next intermoult, to fail at some future moult. A few eyestalkless animals lived longer than 10 days after their final moults. This may possibly be because they were in such a weakened state that the next (incipient) moults were considerably delayed.

Summary

1. Removal of both eyestalks causes a shortening of the following intermoults in *Cambarus clarkii*. The removal of a moult-inhibiting substance produced in the eyestalks is accepted as a reasonable explanation.

2. The methods employed have been such as to show: (*a*) that the processes leading up to moulting can be initiated in the absence of the eyestalks; and (*b*) that the effect upon moulting is associated with the absence of the eyestalks, not with the shock of their removal.

3. Injury other than eyestalk removal does not shorten the intermoult when performed early in the intermoult.

4. Eyestalk removal always results in death, but the processes related to viability which are affected by eyestalk removal can be distinguished from the moulting processes which are affected by the same operation, although there is an apparent correlation between moulting and viability.

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